

**38\* Non-visualization of the gallbladder: a major risk of cystic fibrosis when associated with echogenic bowel**

I. Duguépéroux<sup>1</sup>, V. Scotet<sup>1</sup>, M.-P. Audrézet<sup>2</sup>, A.-H. Saliou<sup>3</sup>, M. Blayau<sup>4</sup>, S. Schmitt<sup>5</sup>, A. Kitzis<sup>6</sup>, C. Fèrec<sup>1,2</sup>. <sup>1</sup>CHU de Brest, Inserm U613, Brest, France; <sup>2</sup>CHU de Brest, Lab. of Molecular Genetics, Brest, France; <sup>3</sup>CHU de Brest, Dpt. of Gynecology & Obstetrics, Brest, France; <sup>4</sup>CHU de Rennes, Lab. of Molecular Genetics, Rennes, France; <sup>5</sup>CHU de Nantes, Lab. of Molecular Genetics, Nantes, France; <sup>6</sup>CHU de Poitiers, Lab. of Molecular Genetics, Poitiers, France

Cystic fibrosis (CF) can be diagnosed in several ways. In utero, several clues may suggest it, the most common being the presence of a foetal echogenic bowel (FEB) detected at ultrasound during pregnancy. Other elements, as nonvisualized foetal gallbladder (NFG) are also possible evidences for CF.

Based on the experience of 4 university hospitals (Brest, Rennes, Nantes, Poitiers) of western France, this study reports the data of NFG, focused on CF, and assesses the absence of gallbladder as a component in achieving a CF diagnosis.

We reviewed all the consecutive cases of NFG in pregnant women and who were referred for an analysis of the gene responsible for CF (CFTR) over the period 2002–2009.

Over that period, 38 NFG were recorded. We identified 5 CF fetuses (F508del/F508del n=2, F508del/937insT n=1, F508del/4005+1G>A n=1, F508del/R553X n=1) and 3 heterozygous ones. This led respectively to a CF prevalence of 13.2% (1/7.5) and a carrier rate of 7.9% (1/12.6) in NFG fetuses. The first rate was significantly higher than in the global population (1/2 928). For all CF fetuses, NFG was associated with FEB, whereas heterozygous patients showed only NFG. All affected pregnancies were terminated.

We highlighted the importance of viewing or not the gallbladder during pregnancy. In our study, NFG seems to be an essential element to consider in achieving a diagnosis of CF, especially when associated with FEB (all the CF fetuses have both).

Support: Programme Hospitalier de Recherche Clinique.

**39\* A comprehensive genotype–phenotype description of 695 cases with fetal bowel anomalies**

A. de Becdelièvre<sup>1</sup>, C. Costa<sup>1</sup>, J.M. Jouannic<sup>2</sup>, A. Le Floch<sup>1</sup>, I. Giurgea<sup>1</sup>, J. Martin<sup>1</sup>, R. Médina<sup>1</sup>, B. Boissier<sup>1</sup>, C. Gameiro<sup>1</sup>, C. Alberti-Boulmé<sup>3</sup>, M. Goossens<sup>1</sup>, E. Girodon<sup>1</sup>. <sup>1</sup>Hôpital Henri Mondor, Genetics, Creteil, France; <sup>2</sup>Hôpital Trousseau, Obstetrics, Paris, France; <sup>3</sup>Hôpital Robert Debré, Epidemiology, Paris, France

Fetal bowel hyperechogenicity and loop dilatation that are observed at the second trimester of pregnancy can be due to several disease conditions, including CF. Screening for frequent CF mutations is performed in a first row and is followed, in particular cases, by an in-depth study. We collated data on 694 referrals related to suspicion of CF in fetuses from 1992 to 2009, in an attempt to provide comprehensive description of CFTR genotypes, correlations with ultrasound signs and risk reappraisal. A group of 465 first-hand referrals was distinguished from another of 229 second-hand referrals that had a first study in another laboratory.

In the 1<sup>st</sup> group, CF was found in 2.1% of fetuses, CFTR-RD in 0.6% and simple heterozygosity in 3%. The residual risk of CF in fetuses carrying one frequent mutation, evaluated from data of the 2 groups, was assessed at 20%. In total, 5 large deletions were identified, of which 3 were new, accounting for 10.3% of CF alleles.

Correlation with ultrasound signs revealed a significant higher frequency of multiple digestive signs in CF (55%), such as hyperechogenic bowel, loop dilatation and non-visualization of gallbladder, than in non-CF fetuses (20%).

In conclusion, this study shows heterogeneity of CFTR genotypes associated with varied combinations of bowel anomalies, leading to a CF or CFTR-RD diagnosis, as is observed for CF newborn screening. It also strongly strengthens the need to consider large CFTR gene rearrangements in the diagnosis strategy.

**40\* Molecular basis of cystic fibrosis in Spanish population**

M.D. Ramos<sup>1</sup>, F.J. Giménez<sup>1</sup>, T. Casals<sup>2</sup>. <sup>1</sup>Idibell, Centre de Diagnosi Genètica Molecular de Malalties Hereditàries (CDGM), Barcelona, Spain; <sup>2</sup>Idibell, Centre de Diagnosi Genètica Molecular de Malalties Hereditàries (CDGM) Centre de Genètica Mèdica i Molecular (CGMM), Barcelona, Spain

From 1988 we have recruited 1,000 CF patients/families for genetic testing. In our current strategy, CFTR gene study is carried out in successive steps: 1) commercial kit for the detection of common mutations; 2) microsatellite analysis (IVS8CA, IVS17bTA) to determine those haplotypes associated to another common mutation or large rearrangement already identified in the Spanish patients and/or to detect loss of heterozygosity; 3) CFTR gene scanning for samples yet uncharacterized using single strand conformation polymorphism (SSCP/HD) and denaturing gradient gel electrophoresis (DGGE) techniques followed by sequencing of abnormal patterns; 4) MLPA analysis to detect rearrangements in samples with yet CF unknown alleles. Any abnormal MLPA profile is confirmed by direct analysis or quantitative real-time PCR. When necessary, other complementary studies are performed: *in silico* analysis, array-CGH to characterize novel rearrangements and/or RNA analysis. Moreover, segregation study is carried out to confirm the CF genotype. Genetic counselling, carrier detection and prenatal diagnosis are offered to the families. Overall, we have identified above 200 mutations with a detection rate of 97%. The F508del accounts for 51% of CF alleles and other 11 point mutations show frequencies  $\geq 1\%$ . Eight large rearrangements account for 1.3%. Our results confirm the high molecular heterogeneity of CFTR gene in the Spanish population just as it has been described in countries of the Mediterranean area.

Supported by: ISCii FIS PI080041 and Fundación Sira Carrasco (Spain).

**41 R117H homozygosity and the genotype–phenotype relationship**

I. Bronsveld<sup>1</sup>, R.A. de Nooijer<sup>1</sup>, H.G.M. Arets<sup>1</sup>, F. Teding van Berkhout<sup>1</sup>, C.K. van der Ent<sup>1</sup>, Y. de Rijke<sup>2</sup>, H.R. de Jonge<sup>3</sup>. <sup>1</sup>University Medical Center Utrecht, Utrecht, Netherlands; <sup>2</sup>Erasmus MC – Sophia Children's Hospital, Clinical Chemistry, Rotterdam, Netherlands; <sup>3</sup>Erasmus MC, Biochemistry, Rotterdam, Netherlands

**Introduction:** The CFTR gene has been cloned now 20 years ago, however we still experience difficulties in diagnostic cases with atypical presentation and complex mutations like R117H. This mutation is relevant in our clinic since a large proportion of persons with positive NBS test by IRT carry a R117H mutation. In our first period of neonatal screening using IRT, PAP and DNA analysis we found R117H in 7 out of 18 CF patients (40%), compared to around 1–5% in CF patients diagnosed on clinical grounds.

**Results:** We diagnosed one male (age 35) and one female (age 33) carrying two R117H (7T) mutations. The male patient presented with infertility because of CBAVD. The female was only recognised because she underwent CFTR mutation analysis after her son was identified with F508del/R117H by the NBS. She had no clinical symptoms. Further characterisation showed borderline sweat tests (Cl<sup>-</sup> 34 and 42 respectively), normal sodium and chloride transport in respiratory epithelium and pancreatic sufficiency.

**Conclusions:** These two homozygous R117H patients emphasize the complex genotype-phenotype relationship. Our male patient only presented with CBAVD and a borderline sweat test. In addition, in both cases NPD was normal. To our knowledge this is a first report on an adult female R117H-7T homozygous individual, she was without any CF symptoms and only had a borderline sweat test. Therefore, we suggest that R117H is associated with vas deference dysfunction in males, but not with any CF symptoms, as was seen in compound heterozygous patients with R117H-7T (Peckham *et al* 2006). We will further investigate the genotype-phenotype relationship in R117H patients identified by the NBS tests.